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Antidepressant properties of the triazolobenzodiazepines alprazolam and adinazolam: studies on the olfactory bulbectomized rat model of depression

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1 Chronically administered alprazolam and adinazolam attenuated the hyperactivity of bilaterally bulbectomized rats when placed in a stressful, novel environment ('open field' apparatus). These drugs had no effect on the activities of sham operated animals under the same experimental conditions. In other studies in these laboratories, clinically effective antidepressant drugs have been shown to have a qualitatively similar effect to alprazolam and adinazolam.

2 Chronically administered diazepam and phenobarbitone did not affect the hyperactivity of bulbectomized rats in the 'open field' apparatus.

3 No difference could be found between the behaviour of bulbectomized rats and the sham operated controls when the animals were placed in a novel, non-stressful environment ('hole board' apparatus and Y-maze). Chronic treatment of either the lesioned or non-lesioned animals with alprazolam or adinazolam did not cause any change in the behaviour of the animals in these situations. This suggests that the behaviour of the rat on the 'hole board' is not a reliable indication of anti-anxiety activity for chronically administered benzodiazepines.

4 When unstarved lesioned and non-lesioned animals were given a choice of five palatable foods for a period of 1 h, slight differences in preference for the type of food chosen could be detected. Thus unsweetened biscuit ('cream crackers') was the most preferred choice of the sham operated rats while cheese and chocolate were the least preferred. Bulbectomized rats showed a more varied food choice, with processed meat ('corned beef') and raisins being preferred to biscuit in two out of four groups. Chronic treatment with either alprazolam or adinazolam did not appear to affect the food preference.

5 Detailed studies of the effects of chronic alprazolam and adinazolam treatment on the steady state concentrations of noradrenaline, dopamine and 5-HT in the amygdaloid cortex and mid-brain regions showed that only the highest, and sedative, dose of alprazolam reduced the 5-HT concentration in the amygdaloid cortex of the bulbectomized animals. Chronically administered diazepam and phenobarbitone also decreased the 5-HT concentration while adinazolam was without effect. No change in the 'turnover' of the biogenic amines could be detected following the chronic administration of these drugs.

6 Despite this apparent lack of effect of alprazolam and adinazolam on the concentrations of brain monoamines, studies by others have shown that those drugs reduce the activity of cortical β-adrenoceptors and enhance those of 5-HT receptors in

the limbic system. This suggests that the triazolo benzodiazepines have a neuropharmacological profile which is qualitatively similar to that of other atypical antidepressants.

Keywords alprazolam adinazolam antidepressants

Introduction

Although there is evidence that such 1, 4-benzodiazepines as diazepam relieve the symptoms of anxiety in patients suffering from mixed anxiety-depression states, there is no evidence that such drugs are useful in treating the core symptoms of depression (Schatzberg & Cole, 1978). Unlike the widely used tricyclic antidepressants, benzodiazepines have a low incidence of serious side effects and lack anticholinergic activity. However, they are liable to abuse and both physical and psychological dependence to commonly used anti-anxiety benzodiazepines have been reported (Marks, 1978; Tyrer, 1983); they also potentiate the central depressant actions of ethanol and other sedatives which can result in the death of the patient.

The triazolobenzodiazepines differ from the classical 1,4-benzodiazepines in that the triazolo ring is incorporated into the benzodiazepine ring. This structural modification results in a reduction in the $t_{1/2}$ of the molecule due to the enhanced rate of absorption and elimination (Abernethy *et al.*, 1983); unlike the 1, 4-benzodiazepines which are largely converted to active metabolites *in vivo*, the triazolo-derivatives appear to be largely devoid of active metabolites. The structures of diazepam and the two triazolo-benzodiazepines investigated in the present study are shown in Figure 1.

Several triazolobenzodiazepines were shown experimentally to have an anti-anxiety profile which was qualitatively similar to diazepam (Rudzik *et al.*, 1973). Of the triazolo derivatives tested, alprazolam was found to be one of the most active compounds in suppressing foot-shock induced aggressive behaviour in mice. The potent antianxiety effects of alprazolam, and the closely related analogue, adinazolam, is probably related to the high affinity which these drugs show for the benzodiazepine receptors (Sethy *et al.*, 1983). In a placebo controlled, double-blind study alprazolam was shown to be an effective antianxiety agent (Aden & Theiss, 1980; Cohn, 1981).

In an open study of alprazolam in a group of out-patients suffering from neurotic depression, Fabre (1976) reported a moderate to marked improvement in 80% of the patients, an effect which was not attributed to its antianxiety activity. Subsequent studies of this drug on the sleep profile of volunteers showed that it had effects which resembled those of tricyclic antidepressant and monoamine oxidase inhibitors. The antidepressant activity of alprazolam has recently been confirmed in a double-blind placebo controlled trial against imipramine by Feighner *et al.* (1983), while preliminary studies by Pyke *et al.* (1983) have shown that adinazolam is also a clinically effective antidepressant. Like the atypical antidepressant mianserin, the antidepressant activity of alprazolam and adinazolam was not predicted from the pharmacological studies in the laboratory. Thus there was little evidence to indicate that these drugs inhibited the re-uptake of biogenic amines into brain tissue either *in vitro* or *in vivo*, an effect shown by tricyclic antidepressants and most 'second generation' antidepres-

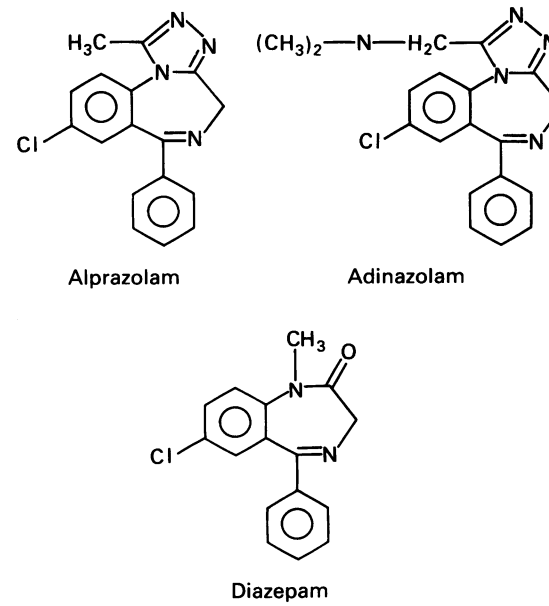

Figure 1 Formulae of alprazolam, adinazolam and diazepam.

sants such as trazodone and nomifensine which lack the tricyclic antidepressant structure. Neither alprazolam nor adinazolam inhibit monoamine oxidase activity. The aim of the present study was to examine the effects of these two drugs on the behaviour of the bilaterally bulbectomized rat. Over the past decade, Cairncross and others have shown that the behavioural deficits produced by bulbectomy can be reversed following the chronic, but not the acute, administration of clinically effective antidepressants irrespective of their structure or acute action on biogenic amine function (Cairncross *et al.*, 1978; Van Riezen *et al.*, 1976); psychotropic drugs which lack antidepressant activity are devoid of activity in this model. Detailed studies in this laboratory have shown that the dose required, and the duration of treatment necessary for the reversal of the behavioural deficit produced by bulbectomy, approximates to that found clinically (Leonard, 1982). Details of the physiological, biochemical and pharmacological aspects of the olfactory bulbectomized rat model of depression have been reviewed elsewhere (Leonard & Tuite, 1981; Jancsár & Leonard, 1981, 1983).

Methods

In all these experiments, male rats of the Sprague Dawley strain (initial weight 350–400 g) were used. All animals were handled by the experimenter several times daily for 3–4 days before the commencement of any of the experiments. Previous studies have shown that such a precedure was essential if the irritability of the animals following surgery was to be minimized and the variance in the subsequent behavioural response reduced. In all experiments, the animals were weighed every 3 days and any changes in their gross behaviour recorded.

Bilateral olfactory bulbectomy was performed under tribromoethanol induced anaesthesia as described in detail elsewhere (Jancsár & Leonard, 1981). Following the removal of the olfactory bulbs by suction, the scalp was sutured and the animals allowed to recover for 14 days before the commencement of chronic drug treatment. Sham operated controls were prepared in the same way as the bulbectomized animals but the olfactory bulbs were left undamaged. Four rats were placed in a cage and each cage consisted of two bulbectomized and two sham operated animals. The following experiments were performed on groups of 7 or 8 rats:-

Effects of adinazolam and alprazolam on the behaviour of bulbectomized rats in the 'open field' apparatus.

Sham operated and bulbectomized rats were injected twice daily (09.00 and 18.00 h) with alprazolam or adinazolam in the doses shown in Tables 2 and 3 for a period of 12 days. At 09.00 h on the 13th day, the animals were placed singly in the centre of the 'open field' apparatus of dimensions given by Gray & Laljee (1971) which was illuminated by a 60 W bulb positioned immediately above the centre of the apparatus. The number of squares crossed (ambulation score), the number of times the animals reared (fore paws raised from the floor) the number of times the animal groomed itself and the number of faecal boli deposited in a 3 min period of observation were recorded. Sham operated and bulbectomized rats which had been injected twice daily with the vehicle (1% Tween 80 in saline) were used as the appropriate controls.

Effects of adinazolam and alprazolam on the behaviour of the bulbectomized rat on the 'hole board' apparatus

Details of the apparatus used have been described by Davies & Wallace (1976). Following chronic drug or vehicle treatment, the number of times the animals crossed the partitions which separated the holes on the surface of the apparatus (crossings), the number of times they explored the holes (head dips), the number of times they groomed themselves and the number of faecal boli deposited in a 3 min observation period were recorded.

Effects of adinazolam and alprazolam on the behaviour of the bulbectomized rat in the Y-maze

The apparatus used was essentially similar to that described by Rushton & Steinberg (1963) with the exception that a hole of approximately 2 cm diameter was placed at the end of each limb of the Y-maze. Following chronic drug or vehicle treatment, rats were placed singly at the junction of the three arms of the maze and the number of times they reared, explored the holes and the number of faecal boli produced in a 3 min observation period were noted.

Effects of adinazolam and alprazolam on food choice

Fourteen days after the commencement of drug

treatment, the home cages containing two bulbectomized and two sham operated rats were partitioned into four areas of equal size one rat being confined to each section. Five small plastic containers were mounted on the wall of each partition; 6–7 g of cheese, raisins, chocolate, processed meat (corned beef) and unsweetened biscuit (cream crackers) were added to these containers. The rats were unstarved and exposed to the different types of food for 1 h daily on 3 consecutive days. For the first 2 days of the experiment, the food eaten from each of the containers was not determined; this enabled the animals to become accustomed to the novelty of the food choice situation. On the third day, the amount of food eaten from each of the containers was determined. The consumption of the different types of food was expressed as a percentage of the total food eaten during the 1 h period.

Changes in brain monoamine concentrations following chronic adinazolam and alprazolam administration

Sixteen days after the commencement of drug administration, the drug treated sham and bulbectomized rats, and their appropriate controls, were decapitated. The brains were rapidly removed, placed on ice and dissected by hand into the amygdaloid cortex and mid-brain by the method of Popov *et al.* (1967). The brain regions were rapidly added to an acid medium and sonicated for 30 s, care being taken to ensure that the temperature of the homogenate did not exceed 20°C. Following centrifugation, the clear supernatant fraction was passed through Sephadex G-10 microcolumns and the amines determined spectrophoto-fluorimetrically by the method of Earley & Leonard (1978).

To assess the effects of these drugs on the 'turnover' of dopamine, noradrenaline and 5-HT in the amygdaloid cortex and mid-brain, groups of seven rats (both sham operated and bulbectomized) were injected with the monoamine oxidase inhibitor pargyline (100 mg/kg) 1.5 h before the animals were sacrificed and 12 h after the last dose of alprazolam or adinazolam. The concentrations of noradrenaline, total 3-methoxy-4-hydroxy phenyl glycol, 5-HT, 5-hydroxy-indole acetic acid and dopamine were estimated fluorimetrically by the method of Earley & Leonard (1978). The aim of this experiment was to determine whether the rise in the concentration of the biogenic amines in the drug treated sham operated and bulbectomized groups differed.

Drugs used

Alprazolam and adinazolam were obtained from Upjohn Ltd, Kalamazoo, U.S.A. The drugs were suspended in 1% Tween 80 in saline and sonicated for 1 min before being injected. Diazepam was obtained in a commercially available injectable form; the appropriate diluent was used as the vehicle for the control injections. Pargyline was obtained from Sigma Chemical Co.

Statistical analysis

All results are expressed as the mean ± s.e. mean and a comparison of the various treatment groups with the appropriate controls made using the two-tailed Student's *t*-test.

Results

Effects of adinazolam and alprazolam on the behaviour of the bulbectomized rat in the 'open field' apparatus

The results are summarized in Tables 1 and 2. In both experiments, it can be seen that the activity of the bulbectomized rat is significantly increased compared to the sham operated controls; the rearing score was also increased significantly in these experiments. Pre-treatment with the lowest doses of the triazolobenzodiazepines resulted in a slight but statistically non-significant reduction in the ambulation scores (8% following alprazolam and 9% following adinazolam); the highest doses of these drugs produced a reduction in the ambulation score (15% for both alprazolam and adinazolam) without significantly affecting the enhanced rearing behaviour of these animals. The activities of the sham operated and bulbectomized rats following drug treatment were also assessed at 1 min intervals to see if there were any changes in the rate of habituation to the 'open field' apparatus between the controls and drug treated groups. No significant differences were found. The effects of diazepam and phenobarbitone on the behaviour of the bulbectomized rat is summarized in Table 3 where it can be seen that the benzodiazepine increased (29%) the hyperactivity of the lesioned animal, whereas phenobarbitone, in a sub-hypnotic dose, had no effect.

Table 1 Behaviour of sham operated and bulbectomized (OB) rats in the 'open field' apparatus following chronic treatment

	Ambulation	*Rearing*	*Grooming*	*Defaecation*
		Alprazolam (2.5 mg/kg twice daily)		
Sham	101 ± 6.9	13.8 ± 4.0	1.85 ± 0.6	1.7 ± 0.8
Sham + drug	107 ± 17	11.2 ± 3.0	1.14 ± 0.3	1.54 ± 0.6
OB	143 ± 17*	25.5 ± 1.5*	1.16 ± 0.65	5.5 ± 1.5
OB + drug	132 ± 10	19.2 ± 2.6*	1.85 ± 0.8	5.4 ± 1.2
		Adinazolam (1.5 mg/kg twice daily)		
Sham	101 ± 9.4	11.0 ± 1.6	1.00 ± 0.3	2.2 ± 1.0
Sham + drug	88 ± 7.0	10.0 ± 0.6	1.20 ± 0.3	2.5 ± 0.9
OB	136 ± 10*	24.0 ± 4.6*	1.30 ± 0.7	4.2 ± 1.0
OB + drug	124 ± 15	23.0 ± 3.6*	1.80 ± 0.7	6.2 ± 1.0*

Alprazolam or adinazolam administered to the appropriate drug treated groups for 21 days in doses shown; $n = 7$ per group. Non drug treated groups injected with vehicle. Animals observed in 'open field' apparatus for 3 min. All values expressed as mean ± s.e. mean.
* $P < 0.05$ *vs* sham.

Table 2 Behaviour of sham operated and bulbectomized (OB) rats in the 'open field' apparatus following chronic treatment

	Ambulation	*Rearing*	*Grooming*	*Defaecation*
		Alprazolam (5.0 mg/kg twice daily)		
Sham	117 ± 14	16 ± 3	2.0 ± 0.5	1.0 ± 0.4
Sham + drug	118 ± 5	25 ± 2*	2.0 ± 0.8	2.0 ± 0.5
OB	134 ± 7*	19 ± 3	2.0 ± 0.6	3.0 ± 0.6
OB + drug	115 ± 10	17 ± 2	3.0 ± 0.6	3.0 ± 0.6
		Adinazolam (5.0 mg/kg twice daily)		
Sham	96 ± 11	12 ± 2	3.0 ± 0.7	2.0 ± 0.7
Sham + drug	104 ± 7	15 ± 2	4.0 ± 1.0	2.0 ± 0.3
OB	126 ± 4*	19 ± 1*	3.0 ± 1.0	3.0 ± 0.5
OB + drug	108 ± 4●	15 ± 2	8.0 ± 2.0*	3.0 ± 0.9

Each group consisted of eight animals; results expressed as mean ± s.e. mean. Alprazolam and adinazolam were administered for 12 days.
* $P < 0.05$ *vs* sham controls.
● $P < 0.05$ *vs* OB control.

Effects of adinazolam and alprazolam on behaviour of the bulbectomized rat on the 'hole board' apparatus

There was a slight increase in the activity of the bulbectomized rats compared to the sham operated animals which was unaffected by chronic adinazolam or alprazolam treatment. The number of crossings for the sham operated controls were 32.0 ± 4.0 while those of the bulbectomized controls were 44.8 ± 4.0; the values for the adinazolam and alprazolam treated bulbectomized groups were 42.0 ± 4.5 and 42.7 ± 3.7 respectively. No change occurred in the head dip score (sham operated group 11.2 ± 1.0), number of groomings in the 3 min observation period (sham operated group 0.28 ± 0.1) or in the number of faecal boli produced (sham operated group, 0.85 ± 0.45). From this study it is apparent that the effects of alprazolam and adinazolam on the behaviour of the bulbectomized rat are only apparent following exposure to a stressful, novel environment as shown by the 'open field' apparatus.

Effects of alprazolam and adinazolam on the behaviour of the bulbectomized rat in the Y-maze apparatus

In this experiment, the number of times the animals reared, explored the holes placed at the end of the arms of the Y-maze (dips) and the number of faecal boli produced in the 3 min observation period was determined. Following

Table 3 Behaviour of sham operated and bulbectomized rats in the 'open field' following chronic diazepam and phenobarbitone treatment

	Ambulation	*Rearing*	*Grooming*	*Defaecation*
		Diazepam (2.5 mg/kg)		
Sham	70 ± 4	10 ± 1	3.0 ± 0.2	2.0 ± 0.4
Sham + drug	98 ± 2*	18 ± 3*	3.0 ± 0.3	3.0 ± 0.5
OB	125 ± 5*	25 ± 6*	4.0 ± 0.6	4.0 ± 0.2
OB + drug	161 ± 5*●	31 ± 7*	3.0 ± 0.2	4.0 ± 0.5
		Phenobarbitone (20 mg/kg)		
Sham	74 ± 8	9 ± 1	1.0 ± 0.3	3.0 ± 0.4
Sham + drug	94 ± 8	15 ± 2*	0.8 ± 0.4	4.0 ± 0.7
OB	149 ± 5*	25 ± 2*	0.8 ± 0.4	4.0 ± 0.6
OB + drug	144 ± 9	24 ± 4*	1.0 ± 0.5	4.0 ± 1.0

Each group consisted of eight animals; results expressed as mean ± s.e. mean. Drugs administered once daily for 12 days.
* $P < 0.05$ *vs* sham controls.
● $P < 0.05$ *vs* OB controls.

the chronic administration of these drugs, no changes were observed in any of these parameters in any of the treatment groups. The number of rears, dips and boli made by the sham operated controls were 9.5 ± 1.4, 5.4 ± 0.61 and 1.4 ± 0.5 per 3 min observation period respectively. Unlike the 'open field' and 'hole board' experiments, the bulbectomized rat did not show any significant difference from the sham operated controls.

Food preference test: effects of alprazolam and adinazolam on the choice of food consumed

The results are summarized in Table 4. The results show the intake of different types of food eaten in a 1 h period. In this experiment, the different types of food had been placed in the cage for 1 h on 3 consecutive days, but the amount of food consumed was only determined on the third day of the experiment. This precedure was adopted as preliminary experiments showed that the sham operated rats initially covered up the food containers with cage bedding and showed little interest in the different types of food. It was of interest to find that the bulbectomized animals did not react in this way. By the third day of exposure to the choice of different types of food however the sham operated rats began to eat some of the foods.

Because of the considerable variation in the food choice of individual rats the standard errors were quite high, in some cases approximating to 40% of the mean value. Under these circumstances, the differences between the drug treated and control groups did not reach statistical significance. However, the following trends appeared:

(a) sham control *vs* OB controls: the OB rats ate less cheese but more chocolate

(b) sham control *vs* sham + alprazolam or adinazolam: the intake of chocolate and corned beef increased while that of 'cream crackers' decreased after alprazolam treatment. By contrast, after chronic adinazolam treatment, only the intake of corned beef increased.

(c) OB control *vs* OB + alprazolam or adinazolam: the change in food intake after alprazolam was qualitatively similar to that seen in the sham operated rats. However, after adinazolam administration to the bulbectomized rats, the intake of cheese and 'cream crackers' increased while that of raisins and chocolate decreased. From this study, it appears that only adinazolam has a qualitatively different effect on the food preference of the bulbectomized rat compared with the sham operated control.

Changes in body weight and gross behaviour following chronic alprazolam and adinazolam treatment

The lowest doses of these drugs did not have an appreciable effect on the behaviour of either the sham operated or bulbectomized rats apart from causing slight sedation for approximately one hour following intraperitoneal injection. By contrast, the highest doses administered caused marked sedation in both groups of animals which persisted for several hours following drug administration. The sedative effect of these drugs was not noticeably diminished after twelve days treatment.

Table 4 Intake of different types of food by sham operated and bulbectomized (OB) rats given choice of diet for 1 h period: effects of chronic alprazolam and adinazolam

	Cheese	*Raisins*	*Chocolate*	*Corned beef*	*Cream crackers*	*Total eaten (g)*
Sham	0.37 ± 0.04 (6%)	1.87 ± 0.82 (28%)	0.72 ± 0.25 (11%)	0.81 ± 0.32 (12%)	2.83 ± 0.48 (43%)	6.60
Sham + alprazolam	0.66 ± 0.18 (8%)	1.62 ± 0.25 (21%)	2.28 ± 0.66 (29%)	1.60 ± 0.59 (20%)	1.69 ± 0.55 (22%)	7.85
OB	0.40 ± 0.04 (14%)	0.31 ± 0.29 (11%)	0.58 ± 0.48 (21%)	0.63 ± 0.18 (23%)	0.78 ± 0.46 (29%)	2.71
OB + alprazolam	0.44 ± 0.11 (9%)	0.72 ± 0.39 (15%)	1.44 ± 0.64 (30%)	1.61 ± 0.69 (34%)	0.55 ± 0.44 (12%)	4.76
Sham	1.61 ± 0.43 (19%)	2.30 ± 0.85 (27%)	1.09 ± 0.52 (13%)	1.46 ± 0.76 (17%)	2.20 ± 0.30 (25%)	8.66
Sham + adinazolam	1.09 ± 0.35 (15%)	1.46 ± 0.87 (20%)	1.00 ± 0.64 (14%)	1.82 ± 0.86 (25%)	1.78 ± 0.59 (25%)	7.15
OB	0.58 ± 0.06 (9%)	1.92 ± 0.49 (31%)	1.73 ± 0.85 (28%)	0.87 ± 0.23 (14%)	1.08 ± 0.53 (17%)	6.18
OB + adinazolam	0.74 ± 0.10 (15%)	0.76 ± 0.40 (15%)	0.88 ± 0.63 (17%)	0.83 ± 0.21 (16%)	1.85 ± 0.62 (37%)	5.06

Type of food eaten (g ± s.e mean) by bulbectomized and sham operated rats (n = 7). Alprazolam and adinazolam administered in doses of 2.5 mg/kg and 1.5 mg/kg twice daily respectively for 12 days. Figures in parenthesis express intake of individual ingredient as percentage of total food intake.

The changes in body weight over the experimental periods have been summarized in Table 5. From these studies it may be concluded that neither alprazolam nor adinazolam significantly affect the growth rate of mature male rats.

Effects of chronic alprazolam and adinazolam administration on steady state concentrations and turnover of biogenic amines

Neither drug had a noticeable effect on the steady state concentrations of noradrenaline, 5-HT or dopamine in either the amygdaloid cortex or mid-brain. In the highest dose administered, alprazolam significantly reduced the rise in the 5-HT concentration in the amygdaloid cortex of the bulbectomized animals (Table 6); adinazolam was without effect and therefore the results are not shown. Under the same experimental conditions, both diazepam and phenobarbitone were found to produce changes in the 5-HT concentrations of the mid-brain and amygdaloid cortex respectively (Tables 7 and 8). Phenobarbitone also reduced the concentrations of noradrenaline and dopamine in the amygdaloid cortex (Table 8). As both phenobarbitone and the triazolobenzodiazepines causes some sedation in the doses used to investigate their effects on the steady state concentrations of the biogenic amines, the study of the effects of alprazolam and adinazolam on amine turnover was conducted using the lower doses 2.5 mg/kg and 1.5 mg/kg twice daily for alprazolam and adinazolam respectively.

Following the administration of the MAO inhibitor pargyline 100 mg/kg to sham operated and bulbectomized rats, the increases in the concentrations of noradrenaline, dopamine and 5-HT were determined 1.5 h later. No significant differences were found between these groups, neither was the chronic administration of alprazolam or adinazolam found to have any significant effect on the rate of increase in the concentrations of the three amine following MAO inhibition. The approximate rates of accumulation of these amines in the two regions studied were:

NA: amygdaloid cortex 0.048 $\mu g\ g^{-1}\ h^{-1}$, mid brain 0.102 $\mu g\ g^{-1}\ h^{-1}$

DA: amygdaloid cortex 0.385 $\mu g\ g^{-1}\ h^{-1}$, mid brain 0.022 $\mu g\ g^{-1}\ h^{-1}$

5-HT: amygdaloid cortex 0.820 $\mu g\ g^{-1}\ h^{-1}$, mid brain 0.890 $\mu g\ g^{-1}\ h^{-1}$

From this study it may be concluded that neither drug affects the turnover of the biogenic amines in their brain regions when chronically administered in non-sedative doses.

Table 5 Changes in body weight following the chronic administration of alprazolam and adinazolam

	Day 1	*Day 17*	*Body weight on:- % increase: day 17 vs day 1*	*Day 37*	*% increase: (day 37 vs 17)*
Experiment 1					
Sham	400 ± 8	428 ± 7	7%	499 ± 10	17%
Sham + alprazolam	394 ± 4	432 ± 8	10%	495 ± 11	15%
OB	376 ± 7	414 ± 8	10%	486 ± 13	17%
OB + alprazolam	375 ± 8	412 ± 10	10%	461 ± 11	12%
Experiment 2					
Sham	405 ± 5	461 ± 3	14%	512 ± 6	11%
Sham + adinazolam	400 ± 5	461 ± 7	15%	521 ± 5	13%
OB	390 ± 5	407 ± 8	4%	456 ± 11	12%
OB + adinazolam	377 ± 5	421 ± 10	12%	472 ± 16	12%

All values expressed as mean weight (g) ± s.e. mean. Sham operated and bulbectomized animals injected with alprazolam (2.5 mg/kg) or adinazolam (1.5 mg/kg twice daily) from day 17; appropriate control groups injected with vehicle.

Discussion

The attenuation of the hypermotility of the bulbectomized rats by alprazolam and adinazolam when the animals were exposed to the novel, stressful environment of the 'open field' apparatus is qualitatively similar to that seen following the chronic administration of both typical and atypical antidepressants (Jancsár & Leonard, 1983). It should be noted however that only the highest dose of adinazolam (5 mg/kg) produced a statistically significant reversal of hypermotility which suggests that these triazolo benzodiazepines are much less potent as antidepressants than they are as antianxiety agents. It is possible that higher doses of adinazolam and alprazolam could have produced a more pronounced effect but the marked sedative action of the highest doses used precluded such a study. It seems unlikely that the changes observed in this study were attributable to non-specific depressant action of the drugs as the sedative hypnotic phenobarbitone and the sedative antianxiety drug diazepam did not attenuate the hypermotility of the bulbectomized rats.

It is clear from the present study that differences between the bulbectomized and sham operated animals only emerge when the rats are exposed to a stressful novel environment. Thus the behaviour of both groups of rats was qualitatively similar when they were placed in the novel non-stressful situation of the Y-maze or on the 'hole board' apparatus. It is noteworthy that chronic treatment of either group with alprazolam or adinazolam did not affect the behaviour of the animals in either of these situations. As the 'hole board' apparatus was developed to assess the potential anti-anxiety activity of psychotropic drugs (Davies & Wallace, 1976) it would appear that its usefulness is restricted to the acute effects of high doses of such compounds. We have previously shown that diazepam (5 mg/kg), when administered for 3 weeks, did not significantly affect any of the parameters measured whereas half this dose administered acutely increased the rearing, crossing and head dip frequency (Kennedy, 1981). It would therefore appear that the 'open field' apparatus is particularly useful for quantifying the difference between bulbectomized and sham operated rats. Undoubtedly, the pronounced stress produced by exposing rats to this environment (O'Connor & Leonard, 1984) accentuates the differences between the lesioned and non-lesioned animals. Despite these differences in the locomotor activities, the rate of habituation of the sham operated and bulbectomized rats to the 'open field' environment was essentially similar.

Studies by other investigators have shown that when rats are placed in an unfamiliar environment and given a choice of familiar and novel foods they will preferentially select the familiar ones (Rolls & Rolls, 1973). On repeating the exposure to the same environment however, the rats develop a preference for the more highly palatable novel foods (Cooper & Crummy, 1978). The present study showed that although the differences in the food choice of the various groups did not reach statistical significance, there were trends to suggest that

Table 6 Effects of chronic alprazolam administration on neurotransmitter concentration in rat brain

	NA		MHPG		5-HT		DA	
	Amygdala	*Mid-brain*	*Amygdala*	*Mid-brain*	*Amygdala*	*Mid-brain*	*Amygdala*	*Mid-brain*
Sham	100 ± 5	100 ± 4	100 ± 6	100 ± 8	100 ± 6	100 ± 10	100 ± 5	100 ± 5
Sham + drug	95 ± 3	94 ± 6	100 ± 7	91 ± 7	92 ± 6	82 ± 4	100 ± 9	90 ± 5
OB	102 ± 2	90 ± 4	92 ± 8	91 ± 9	129 ± 9*	110 ± 9	95 ± 10	104 ± 4
OB + drug	104 ± 5	90 ± 4	107 ± 7	83 ± 10	94 ± •	82 ± 8	100 ± 5	92 ± 5

Each group consisted of eight rats. Values expressed as percentage of the sham controls (= 100%). Absolute values for the sham operated groups were:-
NA: Amygdaloid cortex 0.41 ± 0.02; mid-brain 0.51 ± 0.02; MHPG: Amygdaloid cortex 0.56 ± 0.03, Mid-brain 0.48 ± 0.04; 5-HT: Amygdaloid cortex 0.17 ± 0.01; Mid-brain 0.29 ± 0.03; DA: Amygdaloid cortex 0.20 ± 0.01; Mid-brain, 0.25 ± 0.015. All values expressed as mean ± s.e. mean in μg/g wet weight. Dose of alprazolam used. 5.0 mg/kg twice daily for 14 days.
* $P < 0.05$ *vs* sham controls.
• $P < 0.05$ *vs* OB controls.

Table 7 Effects of chronic diazepam administration on neurotransmitter concentrations in rat brain

	NA		*DA*		*5-HT*		*5-HIAA*	
	Amygdala	*Mid-brain*	*Amygdala*	*Mid-brain*	*Amygdala*	*Mid-brain*	*Amygdala*	*Mid-brain*
Sham	100 ± 15	100 ± 3	100 ± 5	100 ± 6	100 ± 5	100 ± 2	100 ± 2	100 ± 2
Sham + drug	91 ± 10	91 ± 9	110 ± 10	113 ± 11	107 ± 2	75 ± 6*	98 ± 4	102 ± 2
OB	87 ± 4	86 ± 7	95 ± 11	97 ± 7	112 ± 4	102 ± 2	103 ± 5	103 ± 3
OB + drug	91 ± 7	100 ± 6	105 ± 10	93 ± 3	110 ± 5	83 ± 3*•	95 ± 6	102 ± 2

Each group consisted of eight rats; values expressed as a percentage of sham controls (= 100%) Absolute values for sham operated controls: NA: Amygdaloid cortex, 0.32 ± 0.1, Mid-brain 0.35 ± 0.1; DA: Amygdaloid cortex 0.19 ± 0.01, Mid-brain 0.30 ± 0.02; 5-HT: Amygdaloid cortex 0.40 ± 0.02, Mid-brain 0.48 ± 0.01; 5-H1AA: Amygdaloid cortex 0.56 ± 0.01; Mid-brain 0.62 ± 0.01; all values given as μg/g wet weight.
* $P < 0.05$ *vs* sham controls.
• $P < 0.05$ *vs* OB controls.

Table 8 Effects of chronic phenobarbitone administration on neurotransmitter concentrations in rat brain

	NA		*DA*		*5-HT*		*5-HIAA*	
	Amygdala	*Mid-brain*	*Amygdala*	*Mid-brain*	*Amygdala*	*Mid-brain*	*Amygdala*	*Mid-brain*
Sham	100 ± 4	100 ± 4	100 ± 5	100 ± 11	100 ± 6	100 ± 6	100 ± 5	100 ± 2
Sham + drug	80 ± 5*	100 ± 7	73 ± 10*	122 ± 9	77 ± 7*	116 ± 14	111 ± 5	116 ± 8
OB	80 ± 5*	96 ± 8	85 ± 6	122 ± 5	94 ± 6	122 ± 5	97 ± 6	98 ± 7
OB + drug	92 ± 9	122 ± 6	90 ± 5	120 ± 6	72 ± 8*	98 ± 7	110 ± 5	123 ± 2*

Details as shown in legend to Table 6. Absolute values for the sham operated controls were: NA: Amygdaloid cortex, 0.25 + 0.01, Mid-brain 0.27 ± 0.01; DA: Amygdaloid cortex 0.21 ± 0.01, Midbrain 0.18 ± 0.02; 5-HT: Amygdaloid cortex 0.18 ± 0.01, Mid-brain 0.19 ± 0.01; 5-HIAA: Amygdaloid cortex 0.37 ± 0.02, Mid-brain 0.43 ± 0.01. All values given as μg/g wet weight.

* $P < 0.05$ *vs* sham control.

the lesioned animals behaved differently from those of the non-lesioned controls. Thus all groups of sham operated animals showed a preference for unsweetened biscuit ('cream crackers'), a preference which was unaffected by chronic drug treatment, while cheese and chocolate were least preferred. All groups of bulbectomized rats showed a more varied food choice with two of the four groups showing a greater preference for processed meat ('corned beef') and raisins than for unsweetened biscuit; chronic drug treatment did not appear to affect the food preference.

Although these findings do not allow any definitive conclusions to be drawn regarding changes in food preferences which may occur following bulbectomy, they do suggest that refinement of the method used to study food preference may accentuate the differences between lesioned and unlesioned animals. However, as chronic drug treatment does not noticeably affect the food preferences of either the sham operated or bulbectomized rats, the food preference test may primarily reflect a trait rather than state dependent effect of the lesion. It is worthy to note that in the conditioned taste aversion test in which the choice of sweetened water by the bulbectomized rat following an aversive stimulus (LiCl i.p.) differs markedly from that of the unlesioned rat; chronic antidepressant treatment completely reverses the behavioural deficit (Jancsár & Leonard, 1981). This suggests that the differences in food choice indicated by the present study may not be entirely due to differences in taste sensitivity.

Despite the effects of adinazolam and alprazolam on the behaviour of the bulbectomized rat in the 'open field' apparatus which suggest that these compounds have a profile which is qualitatively similar to both typical and atypical antidepressants, these compounds have little apparent effect on the steady state concentrations or turnover (as determined by measuring the steady state concentrations following MAO inhibition) of those biogenic amines which have been implicated in the aetiology of depression. The only statistically significant effect of alprazolam on the steady state concentration of the biogenic amines in bulbectomized rats was found following the chronic administration of the highest dose of the drug when it was found that alprazolam reversed the elevation in the concentration of 5-HT in the amygdaloid cortex. Both diazepam and phenobarbitone were also shown to lower the 5-HT concentration, their effects being apparent in the mid-brain and amygdaloid cortex regions respectively. It thus seems possible that the effect of alprazolam on the brain 5-HT concen-

tration may be attributable to the sedative effect of the dose used; neither adinazolam nor the lower dose of alprazolam had any effect on the steady state concentrations of the biogenic amines. The results of this study therefore suggest that these triazolobenzodiazepines differ from most other antidepressants in their lack of effect on brain biogenic amine turnover.

Regarding the possible mode of action of antidepressants at the cellular level, recent studies have laid emphasis on the dynamic changes in receptor function which occur following the chronic, but not the acute, administration of antidepressants. Thus Vetulani *et al.* (1976) were the first to show that chronic treatment with iprindole, MAO inhibitors and tricyclic antidepressants decreased the functional activity of β-adrenoceptors in the limbic forebrain of the rat. Subsequent studies by this group of investigators showed that all known clinically effective antidepressants and ECT, had a qualitatively similar effect on β-adrenoceptor activity (Sulser, 1978). By contrast to the effects of antidepressants, chronic reserpine treatment was found to increase the activity of β-adrenoceptors in the forebrain. This effect of reserpine has been utilized by Sethy & Hodges (1982) in their investigation of the chronic effect of alprazolam on adrenoceptor function. Thus these investigators showed that alprazolam will attenuate the hyperactivity of cortical β-adrenoceptor in rat brain thereby suggesting that it has a qualitatively similar profile to that of other antidepressants.

With regard to the action of antidepressants on brain 5-HT receptors, chronic treatment with tricyclic antidepressants has been shown by De Montigny & Aghajanian (1978) and by Menkes & Aghajanian (1981) to enhance the responsiveness of rat forebrain neurones to microiontophoretically applied 5-HT. A similar effect was shown by the selective 5-HT uptake inhibitor zimelidine (Blier & De Montigny, 1983) while recently Turmel & De Montigny (1984) have shown that chronic treatment of rats with adinazolam enhanced the responsiveness of hippocampal pyramidal neurons to microiontophoretically applied 5-HT; the responsiveness of these neurons to noradrenaline was unaffected and no change in the 5-HT induced firing rate occurred after acute treatment with adinazolam. From these studies it would appear that chronic treatment with the triazolo benzodiazepines results in a diminished responsiveness of β-adrenoceptors and an enhanced responsiveness of 5-HT (5-HT_2?) receptors. Assuming one can extrapolate from studies on rat forebrain to the brain of the depressed patient, it may be hypothesized that the β-adrenoceptors are hyperactive while the 5-HT receptors are hypoactive. Recent studies on platelet 5-HT receptor activity and β-receptor density on lymphocytes from depressed patients suggest that this is the case (Healy *et al.*, 1983); effective antidepressant treatment is associated with a normalization of the responsiveness of these receptors. It may therefore be concluded that alprazolam and adinazolam are atypical antidepressants in terms of their structure, lack of effect on the reuptake of the biogenic amine neurotransmitters and lack of cardiotoxicity which is generally ascribed to the atropine-like activity of most tricyclic antidepressants. The main difference between the triazolobenzodiazepines and clinically effective atypical antidepressants lies in their potent anxiety-reducing activity. Whether such pharmacological activity will lead to withdrawal effects should drug treatment be abruptly terminated is unknown but preliminary studies by Cohn & Noble (1983) suggest that this might be the case.

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References

Abernethy, D. R., Greenblatt, D. J., Divall, M. & Shader, R. I. (1983). Pharmacokinetics of alprazolam. *J. clin. Psychiat.*, **44**, 45–47.

Aden, G. C. & Thein, S. G. (1980). Alprazolam compared to diazepam and placebo in the treatment of anxiety. *J. clin. Psychiat.*, **41**, 245–248.

Blier, P. & De Montigny, C. (1983). Electro physiological investigations on the effect of repeated zimelidine administration on serotonergic neurotransmission in the rat. *J. Neurosci.*, **3**, 1270–1276.

Cairncross, K. D., Cox, B., Forster, C. & Wren, A. F. (1978). A new model for the detection of antidepressant drugs: olfactory bulbectomy in the rat compared with existing models. *J. pharmac. Meth.*, **1**, 131–143.

Cooper, S. J. & Crummy, Y. M. T. (1978). Enhanced choice of familiar food in a food preference test after chlordiazepoxide administration. *Psychopharmac.*, **59**, 51–56.

Cohn, J. B. (1981). Multicenter double-blind efficacy and safety study comparing alprazolam, diazepam and placebo in clinically anxious patients. *J. clin. Psychiat.*, **42**, 347–351.

Cohn, J. B. & Noble, E. P. (1983). Effect of

withdrawing treatment after long term administration of alprazolam, lorazepam or placebo in patients with an anxiety disorder. *Psychopharmac. Bull.*, **19**, 751–752.

Davies, J. K. & Wallace, P. (1976). The effect of drugs on various parameters of mouse behaviour in a modified head dipping test. *Br. J. Pharmac.*, **57**, 474P.

De Montigny, C. & Aghajanian, G. K. (1978). Tricyclic antidepressants: long term treatment increases responsivity of rat forebrain neurons to serotonin. *Science*, **202**, 1303–1305.

Earley, C. J. & Leonard, B. E. (1978). Isolation and assay of noradrenaline, dopamine, 5-hydroxytryptamine and several metabolites from brain tissue using disposable Bio-Rad columns packed with Sephadex G-10. *J. pharmac. Meth.*, **1**, 67–79.

Gray, J. A. & Laljee, B. (1974). Sex differences in emotional behaviour in the rat: correlation between 'open field' defaecation and avoidance behaviour. *Anim. Behav.*, **22**, 856–861.

Fabre, L. F. (1976). Pilot open label study with alprazolam (U-31, 889) in out patients with neurotic depression. *Curr. Ther. Res.*, **19**, 661–668.

Feighner, J. P., Aden, G. C., Fabre, L. F., Rickels, K. & Smith, W. T. (1983). Comparison of alprazolam, imipramine, and placebo in the treatment of depression. *J. Am. med. Ass.*, **249**, 3057–3064.

Healy, D., Carney, P. A. & Leonard, B. E. (1983). Monoamine-related markers of depression: changes following treatment. *J. Psychiat. Res.*, **17**, 251–260.

Jancsár, S. M. & Leonard, B. E. (1981). The effects of antidepressant drugs on conditioned taste aversion learning of the olfactory bulbectomized rat. *Neuropharmac.*, **20**, 1341–1345.

Jancsár, S. M. & Leonard, B. E. (1983). The olfactory bulbectomized rat as a model of depression In *Frontiers in Neuropsychiatric Research*, eds. Usdin, E., Goldstein, M., Friedhoff, A. J. & Georgotas, A. pp. 357–372. London: MacMillan Press.

Kennedy, B. P. (1981). *An investigation of the neurochemical processes mediating anxiety*. Ph.D. thesis, National University of Ireland.

Leonard, B. E. (1982). On the mode of action of mianserin. *Adv. Biochem. Psychopharmac.*, **31**, 301–320.

Leonard, B. E. & Tuite, M. (1981). Anatomical, physiological and behavioural aspects of olfactory bulbectomy in the rat. *Int. Rev. Neurobiol.*, **22**, 251–286.

Marks, J. (1978). *The benzodiazepines: use, overuse, misuses, abuse*. Lancaster, U.K: MTP Press.

Menkes, D. B. & Aghajanian, G. K. (1981). α_1-adrenoceptor mediated responses in the lateral geniculate nucleus are enhanced by chronic antidepressant treatment. *Eur. J. Pharmac.*, **74**, 27–36.

O'Connor, W. T. & Leonard, B. E. (1984). The effect of stress on the secretion of corticosterone in the bulbectomized rat model of depression. *Ir. J. Med. Sci.*, **153**, 226.

Popov, N., Pohle, W., Rosler, V. & Matthies, H. (1967). Regionale Verteilung von gamma amino Buttersaure, Glutaminsaure, Asparaginsaure, Dopamin, Noradrenalin und Serotonin im Rattenhirn. *Acta med. Biol. Germ.*, **18**, 695–702.

Pyke, R. E., Cohn, J. B., Feighner, J. P. & Smith, W. J. (1983). Open label studies of adinazolam in severe depression. *Psychopharmac. Bull.* **19**, 96–98.

Rolls, E. J. & Rolls, B. J. (1973). Altered food preference after lesions in the basolateral region of the amygdala in the rat. *J. comp. Physiol. Psychol.*, **83**, 248–259.

Rudzik, A. D., Hester, J. B., Tang, A. H., Straw, R. N. & Friis, W. (1973). Triazolobenzodiazepines, a new class of central nervous system depressant compounds. In *The Benzodiazepines*, pp. 285–297. New York: Raven Press.

Rushton, R. & Steinberg, H. (1963). Behaviour of rats in the Y-maze following amphetamine-barbiturate mixtures. *Nature*, **197**, 1017–1018.

Schatzberg, A. F. & Cole, J. O. (1978). Benzodiazepines in depressive disorders. *Arch. gen. Psychiat.*, **35**, 1359–1365.

Sethy, V. H., Russell, R. R. & Daenzler, C. L. (1983). Interaction of triazolobenzodiazepines with benzodiazepine receptors. *J. Pharm. Pharmac.*, **35**, 524–526.

Sethy, V. H. & Hodges, D. H. (1982). Role of β-adrenergic receptors in the antidepressant activity of alprazolam. *Res. Comm. Chem. Path. Pharmac.*, **36**, 329–332.

Sulser, F. (1978). Functional aspects of the norepinephrine receptor coupled adenylate cyclase system in limbic forebrain and its modification by drugs which precipitate or alleviate depression: molecular approaches to an understanding of affective disorders. *Pharmacopsychiat. Neuro-Psychopharmac.*, **11**, 43–52.

Turmel, A. & De Montigny, C. (1984). Sensitization of rat forebrain neurons to serotonin by adinazolam, an antidepressant triazolobenzodiazepine. *Eur. J. Pharmac.*, **99**, 241–244.

Tyrer, P. (1983). Contribution of Round Table Discussion. In *The benzodiazepines: from molecular biology to chemical practise*, ed. Costa, E., pp. 400–406. New York: Raven Press.

Van Riezen, H., Schnieden, H. & Wren, A. F. (1976). Behavioural changes following olfactory bulbectomy in rats. A possible model for the detection of antidepressant drugs. *Br. J. Pharmac.*, **57**, 426–427.

Vetulani, J., Stawarz, R. J., Dingell, J. V. & Sulser, F. (1976). A possible common mechanism of action of antidepressant treatments. *Arch. Pharmac.*, **293**, 109–114.